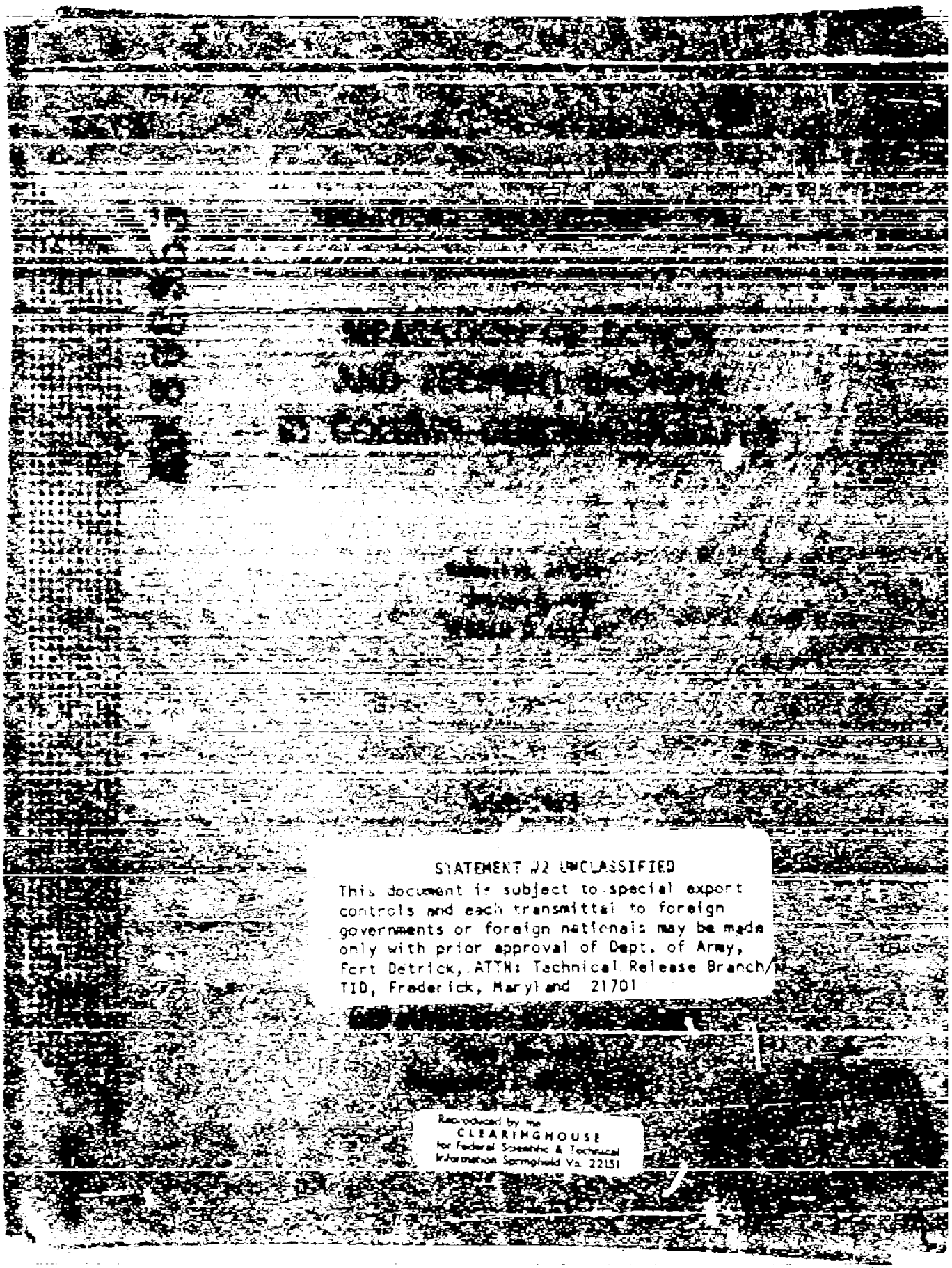

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SEPARATION OF DONOR AND RECIPIENT BACTERIA BY COLUMN CHROMATOGRAPHY

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ABSTRACT

When donor and recipient strains of *Escherichia coli* were added to columns containing Cellex-P (a cation-exchange cellulose), more than 80% of the female cells passed through the column but only 11% or less of the male cells were eluted. However, when donor strains were blended prior to their addition to the column, the majority of these cells were eluted. These results indicated that the filamentous appendages termed F pili (which are removed by blending) were responsible for the adherence of donor cells to the cellulose.

I. INTRODUCTION*

Phenotypic variations are known to exist between the two mating types of bacteria. In 1956, Maccacaro and Cavalli[1] reported that donor and recipient strains of Escherichia coli K-12 differed in their acid agglutination point and in their affinity for basic dyes. In all instances, the recipient (F^-) organisms were found to be more electronegatively charged than the donor (F^+) strains. Subsequently, the existence of the F^+ antigen was demonstrated in Hfr and F^+ strains;[2] this antigen was found to correspond to the small filamentous appendages termed F pili.[3-6] In addition, Smith and Lederberg[7] have demonstrated the existence of a periodate-sensitive site(s) on the surface of Hfr and F^+ cells.

Because these phenotypic differences are expressed at or near the cell periphery and appear to alter the surface charge, we devised a one-step column chromatographic method for separating the two mating types.

II. MATERIALS AND METHODS

A. BACTERIAL STRAINS

The organisms used in this study and their sources are listed in Table 1. Strain W1485 F^- is an acridine orange - cured derivative of W1485 (Falkow). We obtained F^- strains from X^{646} and HB 11 after spontaneous segregation of the F-lac episome.

TABLE 1. ESCHERICHIA COLI STRAINS

Strain	Sex Type	Source
E. coli K12 W1485	F^+	Falkow
E. coli W1485	F^-	Falkow
E. coli W1485	RTF	Falkow
E. coli Hfr Hayes	Hfr	Jacob and Wollman[8]
E. coli X^{646}	F'(pro A^+ pro B^+ lac^+)	Curtiss III
E. coli X^{646}	F^-	Authors
E. coli B HB 11	F'lac	Boyer
E. coli B HB 11	F^-	Authors

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B. MEDIA

Liquid medium was Difco brain heart infusion broth (BHI). Solid media consisted of BHI with the addition of 1.5% agar (Difco) or lactose medium,* to facilitate the assay of F'lac$^+$ and F$^-$(lac$^-$) clones.

C. COLUMN PREPARATION

One gram of Cellex-P (Bio-Rad*), a cation-exchange cellulose, was permitted to swell in 100 ml of 0.1 M ethylenediaminetetraacetic acid (EDTA), pH 6.8. A small piece of glass wool was inserted in the base of a glass column (15 by 300 mm) for support, and 20 ml of the swollen cellulose were transferred to the column and allowed to settle by gravity. The flow rate after packing was approximately 3 ml/min.

D. GROWTH CONDITIONS AND COLUMN ELUTION

Each of the strains listed in Table 1 was grown aerobically for 18 hours in 5 ml of BHI at 37 C. Each culture was diluted with an equal volume of 0.1 M EDTA, pH 6.8, and 1.0 ml of this mixture was added to the column, allowed to drain into the resin, and then followed with 10 ml of 0.1 M EDTA, pH 6.8. The eluate was collected and diluted in 0.15 M NaCl and the appropriate dilutions were plated and incubated overnight at 37 C. Viable counts also were made from a portion of each culture that did not go through the column.

E. RECONSTRUCTION EXPERIMENTS

Equal portions (0.5 ml) of strains X^{646} and X^{646} F$^-$ were mixed in 1.0 ml of 0.1 M EDTA, pH 6.8. One milliliter of this mixture was immediately added to a column and eluted. The eluate was collected and appropriate dilutions were plated on lactose medium.

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III. RESULTS

When individual overnight cultures of female or male bacteria were diluted in 0.1 M EDTA and added to the cation-exchange cellulose, more than 60% of the female cells passed through the column but only 11% or less of the male cells were eluted (Table 2). The only exception was strain W1485 carrying RTF. This result was not unusual because most organisms that harbor R factors are of the repressed type.[10]

TABLE 2. ELUTION OF DONOR AND RECIPIENT BACTERIA FROM CELLEX-P COLUMNS

Strain	Mating Type	Total Colony-Forming Units Recovered		Recovery, %
		Before Column	After Column	
E. coli Hfr Hayes	Male	5.3×10^8	1.5×10^7	2.8
E. coli W1485	Male	1.8×10^9	3.0×10^7	1.7
E. coli W1485 F^-	Female	1.0×10^9	9.2×10^8	92.0
E. coli X^{646}	Male	5.6×10^8	5.5×10^7	9.8
E. coli X^{646} F^-	Female	5.8×10^8	4.9×10^8	84.5
E. coli B HB 11	Male	1.7×10^9	1.9×10^8	11.2
E. coli B HB 11 F^-	Female	2.0×10^9	1.8×10^9	90.0
E. coli W1485 RTF	Male	2.6×10^8	2.1×10^8	80.8

A. SEPARATION OF MATING TYPES

When mixtures of strain X^{646} and X^{646} F^- were added to Cellex-P and eluted, a significant separation of the two mating types was obtained. The results of a typical experiment (Table 3) showed that 90% of the donor cells within the mixture were retained by the column and approximately 90% of the total F^- cells were eluted.

TABLE 3. SEPARATION OF DONOR FROM RECIPIENT CELLS

Strain	Total Colony-Forming Units Recovered from Mixture		Recovery, %
	Before Column	After Column	
X^{646} (*lac*$^+$)	1.4×10^9	1.4×10^8	10.0
X^{646} F^- (*lac*$^-$)	1.7×10^9	1.5×10^9	88.2

B. SPECIFICITY OF DONOR STRAIN

Because periodate treatment is known to interfere with Hfr and F^+ virility by interacting with carbohydrate at the cell surface,[7] periodate might alter the overall charge of the donor cell and change its affinity for Cellex-P. To test this possibility, an overnight culture of Hfr Hayes was treated with periodate according to the procedure described by Smegh and Lederberg[7] before the culture was added to the column. Such treatment did not significantly alter the elution pattern of the donor strain.

C. ROLE OF F PILI

An obvious possibility responsible for the selective retention of donor cells was the F pili produced by donor cells but not produced by recipient cells. Because F pili regeneration takes place readily at 37 C but not at 5 C,[8] an overnight culture of strain W1485 was cooled to and blended at 5 C for 5 minutes in a Sorvall Omni-Mixer, and a sample was added to the resin and eluted. An unblended sample served as a control.

The results of the experiment (Table 4) showed that without blending, 89% of the donor cells were retained by the column, but after blending, only 28% of the donor cells were retained, indicating that the F pili were responsible for the male population adhering to the column.

TABLE 4. EFFECT OF BLENDING ON ELUTION OF *E. COLI* W1485 FROM CELLEX-P COLUMNS

Treatment		Total Number of Colony-Forming Units	
Blended	Column	Recovered	Recovery, %
-	-	3.6×10^8	
-	+	4.0×10^7	11.1
+	+	2.6×10^8	72.2

To determine the viability of donor cells retained by the resin, the Cellex-P-donor mixture was removed from the column with 8 ml of broth and cooled to and blended at 5 C; appropriate dilutions were plated. Recoveries ranging from 70 to 90% were obtained, indicating that Cellex-P had little or no effect on donor viability.

IV. DISCUSSION

The main result of this investigation is discovery of the enrichment of one mating type (F^-) over the other (Hfr, F^+, or F'). When donor and recipient cells were added (individually or in mixtures) to columns containing Cellex-P, 90% of the donor cells were retained by the cation-exchange cellulose. On the other hand, approximately 90% of the recipient cells were eluted.

The elution pattern of donor strains can be altered by high-speed blending, indicating that the male-specific appendages termed F pili are probably responsible for the retention of the males by the column. The F pili may be physically entangled within rather than ionically adsorbed to the cellulose, because increases in salt concentration or changes in pH did not alter the elution patterns of donor bacteria.

Strain W1485 RTF did not exhibit the same retention characteristics as the other donors (Table 2), a result that can be explained on the basis of F piliation. Those organisms that contain a functional episome (one that is derepressed) are capable of producing F pili that, in turn, cause the cells to be retained by the cellulose. On the other hand, organisms that harbor a repressed episome fail to produce these appendages[10] and are eluted. Because of a lack of agglutination of W1485 RTF cells with antiserum to F pili, this strain appears to contain a repressed R factor.

Because Cellex-P appears selectively to separate male cells from female cells (or from repressed male cells), this procedure may prove to be advantageous for (i) isolating rare derepressed cells from a repressed culture, (ii) determining the expression period for F pili formation, and (iii) enriching for recipient cells immediately after they have acquired genetic material from donor strains.

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